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Validation of lethality processes for products with slow come up time: Bacon and bone-in ham

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Abstract
Pork bellies and boneless hams were smoked or cooked using unusually long processes to determine the impact of extended come-up times on the populations of *Clostridium perfringens*, *Salmonella enterica*, *Staphylococcus aureus* and *Listeria monocytogenes*. The products were formulated using brine formulations representative of what might be used in commercial production, and the thermal processes were more than doubled in length. Pork bellies and boneless hams were inoculated on the surface as well as 1 cm below the surface, and samples were collected every 3 h. The populations of *C. perfringens* (spores and vegetative cells) at internal locations of pork bellies increased by less than 1 log10 and declined significantly (approximately 3 log10/cm2) on the surface of the bellies during an extended bacon process. The populations of *S. enterica, L. monocytogenes* and *S. aureus*did not increase during the extended bacon process. The populations of *C. perfringens* (spores and vegetative cells), *S. aureus, S. enterica* and *L. monocytogenes*declined significantly over an extended ham process. There were significant population reductions (>2 log10/cm2) at 7 h (surface) and 12 h (>5 log10/g; internal) for the hams. Populations of both surface and internal locations of the hams declined to a point approaching the limit of detection of the assays within 17 h.

Keywords
bacon, ham, extended processing, Clostridium perfringens, Salmonella enterica, Staphylococcus aureus, Listeria monocytogenes

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Validation of lethality processes for products with slow come up time: bacon and bone-in ham

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ABSTRACT

Pork bellies and boneless hams were smoked or cooked using unusually long processes to determine the impact of extended come-up times on the populations of *Clostridium perfringens*, *Salmonella enterica*, *Staphylococcus aureus* and *Listeria monocytogenes*. The products were formulated using brine formulations representative of what might be used in commercial production, and the thermal processes were more than doubled in length. Pork bellies and boneless hams were inoculated on the surface as well as 1 cm below the surface, and samples were collected every 3 hours. The populations of *C. perfringens* (spores and vegetative cells) at internal locations of pork bellies increased by less than 1 $\log_{10}$ and declined significantly (approximately 3 $\log_{10}/cm^2$) on the surface of the bellies during an extended bacon process. The populations of *S. enterica, L. monocytogenes* and *S. aureus* did not increase during the extended bacon process. The populations of *C. perfringens* (spores and vegetative cells), *S. aureus, S. enterica* and *L. monocytogenes* declined significantly over an extended ham process. There were significant population reductions (> 2 $\log_{10}/cm^2$) at 7 h (surface) and 12 h (> 5 $\log_{10}/g$; internal) for the hams. Populations of both surface and internal locations of the hams declined to a point approaching the limit of detection of the assays within 17 hours.

1. INTRODUCTION

Cured meat products such as bacon or ham are produced by the addition of curing salts (nitrites) and thermal processing. The thermal process for bacon is a smoking process designed to add flavor, and does not result in a fully cooked product, while many of the thermal processes for hams are intended to result in a fully cooked, ready-to-eat product. In the United States, the thermal processes are regulated by the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS 2017a), with the emphasis on the control of non-typhoidal *Salmonella enterica*.

A typical commercial smoking cycle for bacon is in the range of 6 to 8 hours, while the typical commercial cooking cycle for ready-to-eat hams is in the range of 8 to 12 hours. However, these cycles may be extended, either unintentionally through process deviations or by intent, for custom products. While process deviations are unplanned and random, the intentional extension of the thermal cycles are based on the belief that products processed in this way are more flavorful. Although USDA FSIS does not prohibit extended thermal processes, it is incumbent upon the process to demonstrate that they do not create a potential hazard (USDA FSIS 1999).

One of the significant trends in the retail food business is charcuterie (Klein, 2017), which focuses on the production of specialty cured, ready-to-eat products (Rublman and Poleyn, 2005). Some of these products are produced with thermal processes which may be as much as twice as long as the typical commercial processes, and advertised as “slow smoked” or “slow cooked”.

From a food safety perspective, the concern with either an intentional or unintentional extended thermal cycle is the potential change in the populations of

pathogenic bacteria, as these extended cycles may hold the product within the expected growth range of the bacteria. However, a previous study examined the growth potential of *Staphylococcus aureus* and *S. enterica* under isothermal conditions for extended time periods in a model system, and did not note any concerns (Burnham, Fanslau, and Ingham, 2006). This has implications for fully cooked products, as the guidance on thermal processing is based on an assumption of a relatively low initial population of non-typhoidal *Salmonella*. If the extended process resulted in a significant increase in the bacterial population, it is conceivable that the temperatures and times recommended would not be adequate to assure the destruction of the pathogen. This potential increase in bacterial population could be even more significant in a thermal process which was not intended to result in a fully cooked product, and would instead be cooked by the consumer.

An additional concern with a potential increase in population would also be that the guidance on cooling processes may not be adequate (USDA-FSIS 2017b). The guidance is based on controlling the potential survival and growth of *Clostridium perfringens* during the cooling process, and is also based on an assumption that the initial population would be relatively low (Taormina, Bartholomew and Dorsa, 2003). Recently, USDA-FSIS has also raised a concern with the potential growth of *St. aureus* during the cooling process as well (USDA-FSIS 2017b). As with non-typhoidal *Salmonella* and thermal processing, the cooling guidance could be inadequate if there were an increase in the population of *C. perfringens* or *S. aureus*.

The objectives of this study were to determine the potential for changes in microbial populations of foodborne pathogens during extended thermal cycles for bacon

and ham. While no study can examine all of the possible variations of these cycles, this study evaluated thermal cycles that were considered to be at or near the limits of what would be practical during an intentionally extended thermal process.

2. MATERIALS AND METHODS

2.1 Bacterial Cultures: The bacterial strains used in these experiments are described in Table 1. Three different strains of each bacterium were used. *Clostridium perfringens* strains were cultured in fluid thioglycolate medium and in Duncan-Strong sporulation medium (Duncan and Strong, 1968) as described by Juneja, Call and Miller (1993). The cultures grown in fluid thioglycolate medium were used to prepare primarily vegetative cells, while those grown in Duncan-Strong medium were grown primarily to produce spores. The cells and spores were harvested separately by centrifugation (9,500 g, 10 min, 4^{o}C) and were then resuspended in 1/10 volume of physiological saline (0.85% sodium chloride, wt/vol). Prior to inoculation of the pork bellies or hams, the harvested cells and spores were mixed in equal volumes, to prepare an inoculated population that was composed of both vegetative cells and spores.

Staphylococcus aureus, *Salmonella enterica* and *Listeria monocytogenes* were grown to late logarithmic growth stage in trypticase soy broth at 37^{o}C for 18-24 hours. The cultures were harvested by centrifugation as described above, and then re-suspended in 1/10 volume of buffered peptone water.

2.2 Pork bellies and Bone-in hams: The pork bellies and bone-in hams were obtained from the Iowa State University Meat Laboratory, which is a federally inspected establishment. The bellies and hams were processed using brine solutions which were

commonly used in the Meat Laboratory, and similar to those used in commercial practice, although liquid smoke, which may be used in commercial practice, was not included in the brine (Table 2).

2.3 Meat preparation: The pork bellies were injected to 12% of the green weight of the bellies. The injected bellies were tumbled for 30 minutes on a continuous cycle at slow speed. The bone-in hams were injected to 15% of their green weight. The hams were tumbled for 1 hour, covered with the same brine used to inject, and held overnight at 4^{o}C.

2.4 Inoculation and processing: The pork bellies were surface inoculated on the lean side of the belly with a mixed culture of the *C. perfringens* (spores and vegetative cells), *S. aureus, L. monocytogenes* and *S. enterica* with a foam paint brush. The bellies were also inoculated sub-surface by injecting approximately 0.2 ml of the same mixed culture to a depth of 1 cm below the surface on the lean side. The inoculated bellies were covered with plastic to prevent surface drying and stored at 5^{o}C for 72 hours prior to processing. This resulted in inoculated bacteria being in a physiological state which would be typical of those seen in commercial meat processing.

An extended bacon smoke cycle was developed in consultation with industry professionals, University extension faculty and State regulatory personnel to represent an unusually long bacon process. While a commercial bacon process might take 6 to 8 hours, the process evaluated in this study was extended to 15 hours (Table 3). Although designated as a smoke cycle, the smoke unit was turned off for this process, so that only the effects of temperature would be measured.

131 The bone-in hams were inoculated on the surface with the same mixed culture
132 previously described, in a similar manner to that of the bellies. The hams were also
133 inoculated by injecting approximately 0.2 ml of the same mixed culture to a depth of 1
134 cm below the surface on the cushion. The inoculated hams were covered with plastic to
135 prevent surface drying and stored at 5^{o}C for 72 hours prior to processing, again to result
136 in bacteria in a physiological state similar to that seen in meat processing. The hams
137 were processed using an extended cook cycle, developed through consultations as
138 described above. While a typical commercial cooking cycle for bone in hams might take
139 as long as 8 hours, the cycle evaluated in this project was extended to 24 hours. As with
140 the bacon, no smoke was used during the process to assure that only the effects of
141 temperature were measured.
142 2.5 Microbiological analysis: Samples were taken from both the bellies and hams prior
143 to the beginning of the process, at each step during the process and at the end of the
144 process. Surface samples, taken from either the bellies or the hams, consisted of a pre-
145 determined surface area (2x2 or 2x1 cm) aseptically removed with a sterile scalpel and
146 forceps. Internal samples from the hams were obtained by excising the tissue around
147 the injection site to a depth of approximately 2 cm, with the weight of the sample
148 recorded. Samples were homogenized in buffered peptone water and serially diluted as
149 necessary.
150 *C. perfringens* populations were enumerated by surface plating on Perfringens
151 agar with tryptose sulfite cycloserine and egg yolk emulsion (Oxoid, Basingstoke, UK)
152 and incubated at 35^{o}C in anaerobic jars for 48 h. *St. aureus* populations were
153 enumerated by surface plating on Baird-Parker agar with egg yolk tellurite emulsion,

and incubated at 37°C for 48 h. *S. enterica* were enumerated using the thin agar layer method of Kang and Fung (2000) to recover thermally injured cells, with Xylose Lysine Deoxycholate (XLD) agar as the selective layer and trypticase soy agar as the non-selective layer. The plates were incubated at and 37°C for 48 h. *L. monocytogenes* populations were enumerated by surface plating on Listeria selective agar (Oxford) with the modified Oxford supplement and incubated at 37°C for 48 h.

2.6 Experimental Design: Each trial (bellies or bone-in hams) was independently replicated three times, with duplicate samples for each sampling point within replication. Microbial populations were transformed to $\log_{10}$ colony forming units/ cm^2 (surface samples) or g (internal samples). The microbial populations were analyzed by a one-way analysis of variance, with time as the independent factor. Unless otherwise noted, statistical differences were determined at P =0.05.

3. RESULTS

3.1 Pork Bellies: The populations of the inoculated bacteria in or on the pork bellies during the extended bacon process are shown in Fig. 1 and 2 with the net change in population shown in Table 5.

3.1.a Surface Inoculum: The populations of all of the surface-inoculated bacteria declined significantly (P<0.05) during the 15 hour process (Fig. 1). The population of *C. perfringens* declined by 2.8 $\log_{10}$ cfu/cm^2 (P<0.05) during the 15 hour process (Table 5). The population of *St. aureus* also declined over time, with the populations after 15 hours being significantly (P <0.05) less than those between 0 and 6 hours. As with *C. perfringens*, there was approximately a 2.7 $\log_{10}$ reduction during the process. The

177 populations of *L. monocytogenes* and *S. enterica* were both reduced by approximately
178 2.7 $\log_{10}$ reduction during the process.
179 3.1.b Internal Inoculation: The pork bellies were inoculated to a depth of 1 cm with the
180 same mixed culture of bacteria (Fig. 2 and Table 5). The overall trend was for the
181 populations to either remain statistically not different or, in the case of *S. enterica*,
182 decline. The populations of *C. perfringens* showed an increase during the 15 hour
183 process, with a 1 $\log_{10}$ cfu/g increase at 12 hours. However, the population
184 subsequently declined by the end of the process to a 0.7 $\log_{10}$ increase, which was not
185 statistically different ($P>0.10$) from the initial population. The populations of *St. aureus*
186 and *L. monocytogenes* were not statistically different from the initial population over
187 time ($P >0.10$). The populations of *S. enterica* remained relatively constant for the first
188 12 hours ($P>0.10$), but were reduced by approximately 2.3 $\log_{10}$ after 15 hours.
189 3.2 Ham: The results of the surface and internal ham samples are presented in Figure
190 3, with the net change in populations shown in Table 6. The extended process resulted
191 in significant ($P<0.05$) reductions in the populations of all of the bacteria on the surface
192 samples at 7 hours, and on the internal samples after 12 hours.
193 3.2.a Surface Inoculation: The populations of all of the surface-inoculated bacteria
194 declined significantly ($P<0.05$) during the 24 hour process (Fig. 3). *C. perfringens*
195 declined by 2.5 $\log_{10}$ cfu/cm^2 ($P<0.05$) during the extended process (Table 6). The
196 populations of *St. aureus*, *L. monocytogenes* and *S. enterica* also declined over time,
197 with the population reductions after 12 hours of 4 to 5 $\log_{10}$ cfu/cm^2.
198 3.2.b Internal Inoculation: The hams were inoculated to a depth of 1 cm with the same
199 mixed culture of bacteria. As with the surface samples, all of the populations declined

during the extended process ($P<0.05$) in a pattern similar to that seen with the surface populations. *C. perfringens* declined by 1 $\log_{10}$ cfu/cm^2 ($P<0.05$) during the extended process. The population reductions of *St. aureus*, *L. monocytogenes* and *S. enterica* were approximately 4 to 5 $\log_{10}$ cfu/g after 12 hours, and greater than 6 $\log_{10}$ cfu/g after 18 hours. The variation seen with the internal ham samples at 12 hours was, for every bacterium, attributable to one sample with higher populations than the other samples. If that individual sample been removed from the analysis, the variation would have been much less, and significant declines in population would have been noted after 7 hours.

4. DISCUSSION

Much of the research with bacon and ham has focused on the elimination of *S. enterica* during heating (lethality) or on the potential outgrowth of *C. perfringens* during cooling (stabilization). The lethality guidelines published by USDA-FSIS (USDA-FSIS 2017a) are based in part on the research of Goodfellow and Brown (1978), which established the relationship between temperature and time to eliminate *S. enterica*. Later guidance from USDA-FSIS (Decision Risk Consultants, 2005) determined that a thermal process for beef should achieve a minimum 6.5 $\log_{10}$ reduction per gram of non-typhoidal *Salmonella*. While the ham process used in this study met this requirement and would have been considered ready-to-eat but not shelf stable, the smoking process for bacon results in a product which is “ready-to-cook”.

The stabilization guidelines from USDA-FSIS (1998, 2017b) are partially based on mathematical modeling of the outgrowth of *C. perfringens* (Juneja, Marmer and Miller, 1994; Juneja, Huang and Thippareddi, 2006). The guidance states that the stabilization process can allow not more than a 1 $\log_{10}$ increase in the population of *C.*

223 *perfringens* per gram, although with adequate justification a 2 $\log_{10}$ increase may be
224 allowed. The USDA-FSIS guidance is based on an assumption that the initial population
225 of *C. perfringens* spores in raw meat is in the range of 2 – 3 $\log_{10}$ per gram (Taormina,
226 Bartholomew and Dorsa, 2003). The study presented in this paper did not address
227 stabilization. However if a large increase in the population of *C. perfringens* resulted
228 from an extended process, then the stabilization assumptions may need to be re-
229 evaluated.

230 Taormina and Bartholomew (2005) developed a model system to validate bacon
231 processing, which involved the use of both ground pork bellies and pieces of pork
232 bellies. The authors of that study evaluated products with and without liquid smoke, to
233 determine the impact on the outgrowth of *C. perfringens* and *St. aureus*. The samples
234 without liquid smoke in the Taormina and Bartholomew (2005) would be analogous to
235 the samples in the study presented here, as neither liquid smoke or wood smoke was
236 used in the bacon processing.

237 In the Taormina and Bartholomew study, the authors reported increases in the
238 populations of both *C. perfringens* and *St. aureus* in the belly samples without smoke, at
239 the "peak smoking temperature" of 48.9^{o}C, at 6 hours. In the study presented in this
240 manuscript, there was also an increase in the population of *C. perfringens* after 9 and
241 12 hours in the internal belly samples without smoke, although the processing
242 temperatures and times were different between this study and the Taormina and
243 Bartholomew paper. However, unlike the previous study, there was no statistical
244 difference in the populations of *S. aureus* in the internal samples (Fig. 2).

In the present study, the pork bellies and hams were chilled for 72 h after inoculation and prior to processing, which would represent typical commercial processing conditions. Chilling the inoculated product would have resulted in the inoculated bacteria being in lag phase, which would be typical of bacteria during a bacon process, where the bellies are prepared under refrigerated (<10^{o}C) conditions. The bacteria, being in lag phase, would have needed an extended period of time to initiate growth. The impact of this can be seen by manipulating the "physiological state" input on the ComBase non-thermal survival model. For example, the ComBase model predicts no increase in the population of *C. perfringens*, based on a physiological state of approximately 50% of the maximum value. In addition, the surface-inoculated samples become drier during the process. This was particularly evident with the extended processing cycle, where the surfaces were noticeably dehydrated at the end of the cycle. The surface dehydration contributed to the decline in bacterial populations seen primarily at the 12 and 15 hour sampling times. Ingham et al (2004) found that the growth of *S. aureus* in a model ham system in the laboratory generally followed the predictions of the USDA ARS Pathogen Modeling Program.

The results of the ham samples follow established concepts of thermal processing. Integrated (occasionally referred to as accumulated) lethality is the overall lethality of a process on a bacterium, integrated for all of the temperature and time components. That is, there is some lethality attributable to the time a product spends at each temperature, and the overall lethality of the process is the sum of these individual lethality's. The results of the ham experiments certainly demonstrate this.

There has been some concern expressed about the potential for the survival of *C. perfringens* spores during lethality processes, which may potentially grow during stabilization. In these experiments, the *C. perfringens* inoculum was developed to include a very high percentage of spores. The population of *C. perfringens* declined over time in the ham samples, which does not suggest survival of the spores. A reasonable explanation of this data is that the *C. perfringens* spores began to germinate during the extended process cycle. Once germinated, the vegetative cells of *C. perfringens* were susceptible to the thermal lethality of the process.

6. Conclusions: The design of these experiments was intended to measure to possibilities for the growth of specific foodborne bacterial pathogens under extended processing conditions. An important inhibitor commonly used in the processing of bacon and ham (smoke, liquid or wood) was deliberately left out of the formulation and process, in order to assess the impact of the extended process itself. In these experiments, the only increase noted was in *C. perfringens* in the internal pork belly samples, and the increase was not seen until 9 hours into the process. For all of the other bacteria in both the pork bellies and hams, the populations either remained consistent or in many cases showed significant reductions. The results of this study indicate that the potential for large increase in the populations of the four foodborne pathogens is extremely unlikely, even with unusually long processing procedures.

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340 Table 1. Strains of bacteria used to inoculate pork bellies and bone-in hams.

Bacterium	Strains
C. perfringens	ATCC 10258 ATCC 3124 ATCC 12917
S. aureus	Swine Isolate (Iowa State University Veterinary Diagnostic Laboratory) Pork Skin isolate (Iowa State University Veterinary Diagnostic Laboratory) ATCC 29737
S. enterica	Typhimurium ATCC 700720 Montevideo (clinical isolate) Newport ATCC 6962
L. monocytogenes	H7769 H7764 1/2a Scott a

341

343 Table 2. Brine formulations for pork bellies and bone-in hams.

Product	**Ingredient**	**Weight (kg)**
Pork Bellies	Water	36.093
	Salt	5.789
	Sugar	1.930
	Phosphate (Bac O Phos)	1.321
	Sodium Erythorbate	0.208
	Sodium Nitrite	0.045
Bone-In Hams	Water	36.6
	Salt	4.99
	Sugar	2.99
	Phosphate (Cur-A-Phos)	0.635
	Sodium Erythorbate	0.1
	Sodium Nitrite	0.036

344

345

345 Table 3. Extended bacon process cycle.

Step	Time (hours)	Elapsed Time (h)	Step Type	Dry Bulb °C	Wet Bulb °C
1	0	0	-	-	-
2	3	3	cook	40	30
3	3	6	smoke	48.9	37.8
4	3	9	cook	51.7	37.8
5	3	12	smoke	51.7	37.8
6	3	15	finish	57.2	37.8
			Hold to 51.7 °C internal		

346

347 Table 4. Extended cook process for bone-in hams.

348

Step	Time (hours)	Elapsed Time (h)	Step Type	Dry Bulb °C	Wet Bulb °C
1	0	0	-	-	-
2	3	3	cook	60	
3	4	7	cook	65.6	
4	5	12	cook	65.6	57.2
5	5	17	cook	68.3	60
6	4	21	cook	71.1	62.8
7	~3	~24	cook	73.9	68.3
			Hold to 65.6 °C internal		

349

350 Table 5. Net change in populations of *Clostridium perfringens*, *Salmonella enterica*,
351 *Listeria monocytogenes* and *Staphylococcus aureus* on inoculated pork bellies during
352 an extended simulated smoke cycle.

353

Sample Location	*C. perfringens*	*S. enterica*	*L. monocytogenes*	*S. aureus*
	Net Change in Population[A]			
Surface	-2.8	-2.7	-2.7	-2.7
Internal	+0.7	-2.3	-0.2	-0.2

354

355 A Net change in population = initial population, time 0 (log_{10} cfu/g) – final
356 population, end cycle (log_{10} cfu/g)

357

358 Table 6. Net change in populations of *Clostridium perfringens*, *Salmonella enterica*,
359 *Listeria monocytogenes* and *Staphylococcus aureus* on inoculated hams during an
360 extended simulated processing cycle.

361

Sample Location	*C. perfringens*	*S. enterica*	*L. monocytogenes*	*S. aureus*
	Net Change in Population[A]			
Surface	-2.5	-5.8	-6.2	-6.3
Internal	-1.0	-6.0	-0.2	-6.3

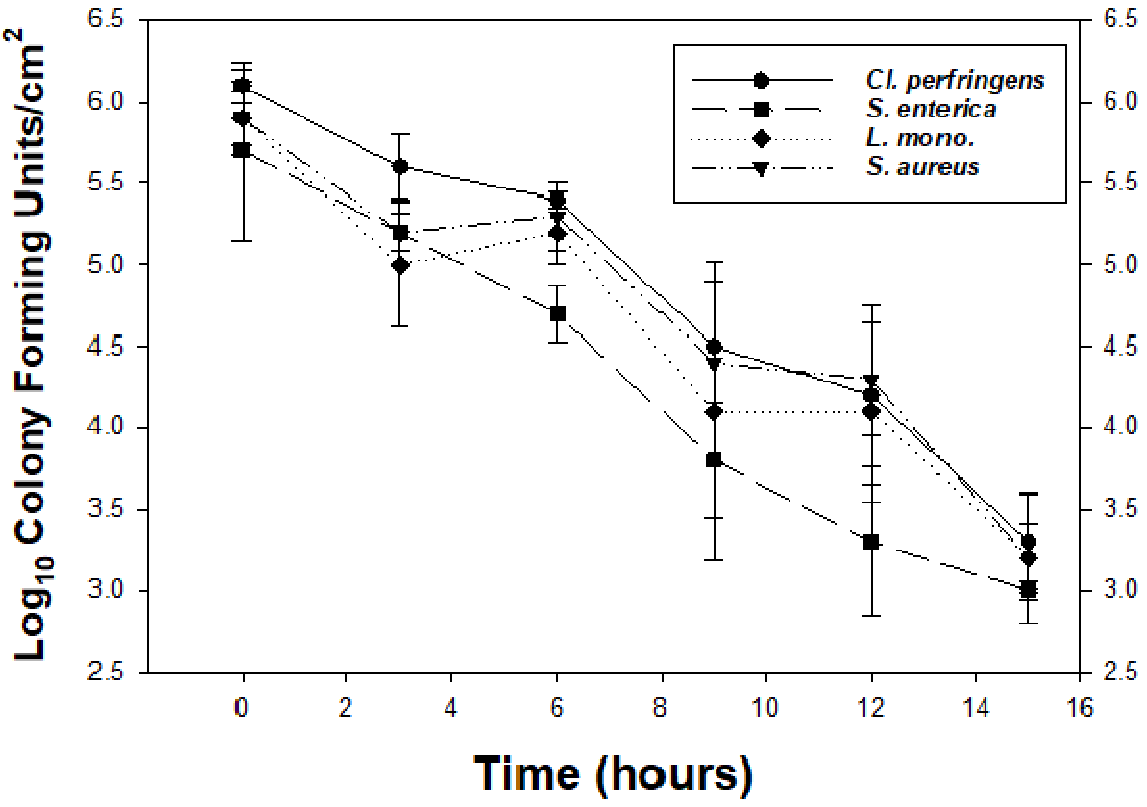
362

363 A Net change in population = initial population, time 0 (log_{10} cfu/g) – final
364 population, end cycle (log_{10} cfu/g)

365

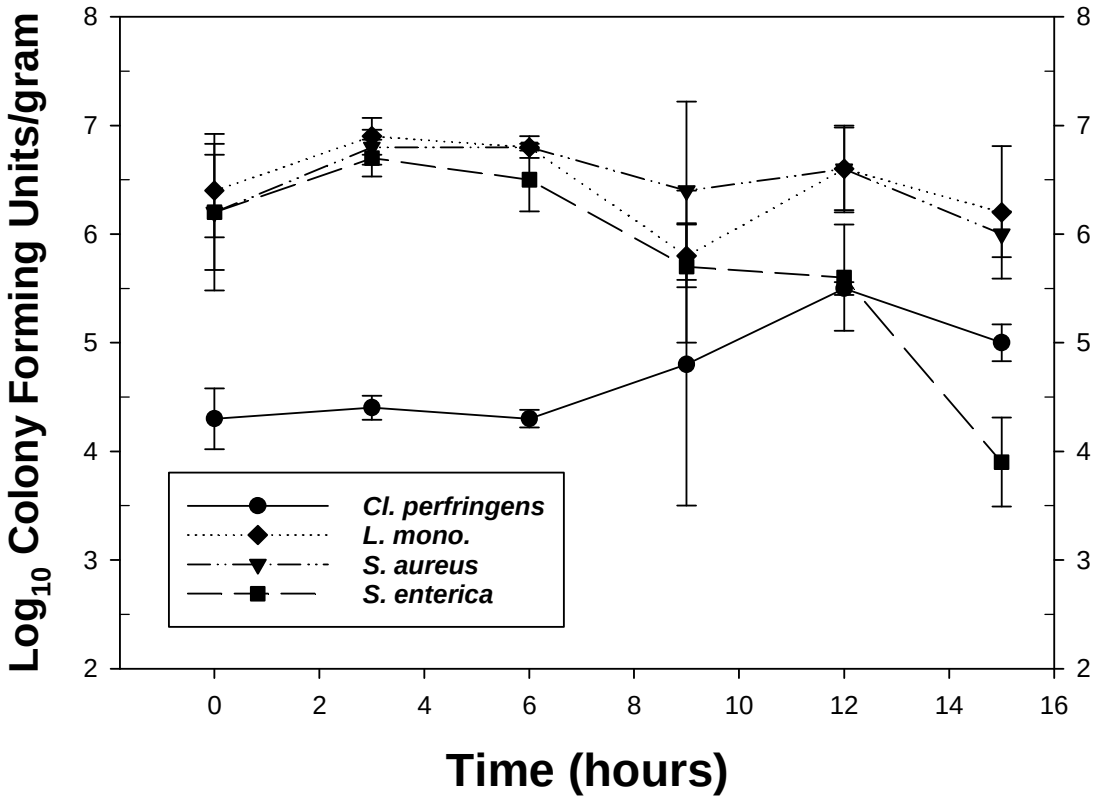
366

365 Figure 1. Populations of *C. perfringens*, *S. aureus*, *S. enterica* and *L. monocytogenes*
366 over time in surface samples of extended cycle bacon.



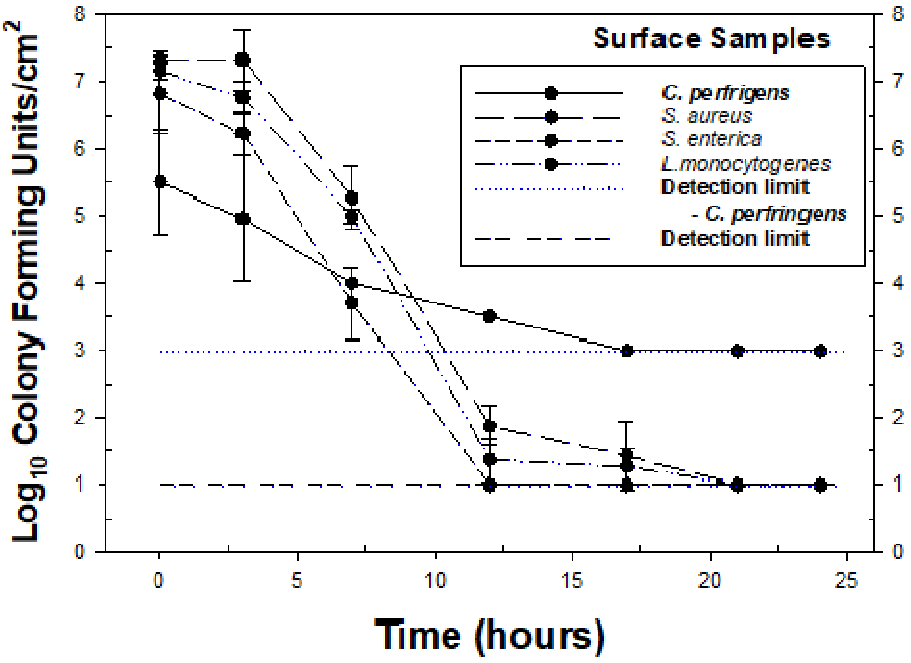
367

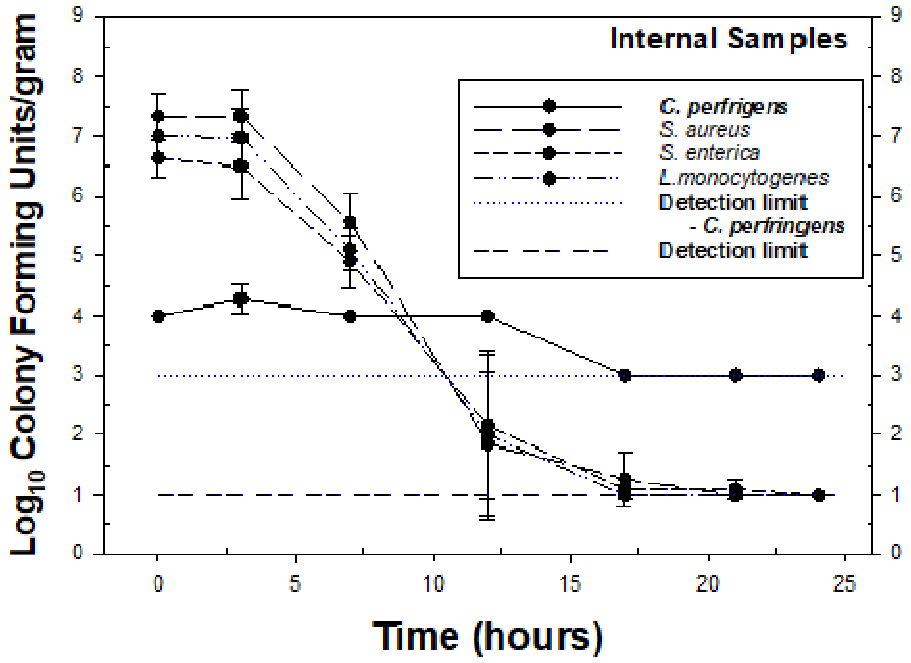
367 Figure 2. Populations of *C. perfringens*, *S. aureus*, *S. enterica* and *L. monocytogenes*
368 over time in internal samples of extended cycle bacon.



369

370 Figure 3. Populations of *C. perfringens*, *S. aureus*, *S. enterica* and *L. monocytogenes*
371 over time in surface and internal samples of slow cooked ham.





372

Validation of lethality processes for products with slow come up time: bacon and bone-in ham

Highlights

C. perfringens increased by less than 1 $\log_{10}$ during an extended bacon process.

S. enterica and *S. aureus* did not increase during an extended bacon process.

L. monocytogenes did not increase during an extended bacon process.

Populations of *C. perfringens* declined significantly during an extended ham process.

The other bacterial pathogens declined significantly during an extended ham process.